SMB

Putamen Ovi

The present invention pertains to a method for the preparation of putamen ovi, putamen ovi having a defined grain size distribution, and to the use of putamen ovi for the treatment of various diseases.

Putamen ovi within the meaning of the present invention comprises hygienically processed egg-shell, in particular from *Gallus domesticus*.

According to Römpp Chemielexikon, 9th edition, 1990, page 1079, item "Eier" ("eggs"), the egg-shell of chicken eggs has a thickness of from 0.2 to 0.4 mm and is white or brown in color, depending on the breed. It is composed of a protein framework (protein-mucopolysaccharide complex) in which calcium carbonate as well as a minor amount of Ca and Mg salts are incorporated. The shell contains pores (7000-17,000 per egg) which are filled with protein fibers. The eggs of other bird species, such as goose, duck, pigeon or quail, are much less important than chicken eggs and are always indicated according to their origins. The shell has a dry mass content in the order of 98.4% which consists of 3.3% of proteins and 95.1% of minerals.

From SU 1 754 104, the use of egg-shells as a dentifrice is known. The use of this preparation is that of a dentifrice. It contains an allegedly caries-inhibiting film with reducing abrasive properties. This dentifrice contains only a very small and non-activity-determining proportion of egg-shells as an adjuvant. The egg-shell powder is not present as a monosubstance,

but is embedded in sodium hydrogencarbonate (35-45% (m/m)) and is not taken up by the organism.

In CH 193 065 A, a liquid tonic is described which is rich in egg yolk, and thus particularly rich in cholesterol, and has high sugar and alcohol contents, but contains little egg-shell components. This is due to the preparation method. The finished emulsion contains from 2 to 3% of egg-shell components in the form of citrates - but only those which are dissolved or emulsified. This preparation contains only particular fractions of egg-shell. Due to its high proportion of cholesterol, sugar and alcohol, this tonic is not acceptable therapeutically in view of its clear potential of load on physiological feedback control systems.

According to FR-A-0 649 055, the egg-shells are sterilized with 20% ethylene oxide at 50°C under a pressure of 5 atmospheres. This method enables a germ reduction rather than a complete sterilization which would be necessary.

The preparation described in GB 2 218 906 is employed for the treatment of dermal lesions. Finely ground egg-shells are processed into a preferably liquid formulation to be used orally or topically which in particular also includes essences, paraffin and various waxs and paraffin oils. The use of this preparation with eczema and allergic skin conditions is not acceptable by oral and topical application since egg-shells, due to their protein base, have a high allergenic potential themselves and may trigger typical skin irritations and increase existing syndromes of certain dermal lesions of allergic nature. Heating the egg-shells for sterilization by means of microwaves over a period of 6 minutes is inadequate for eliminating pathogens.

In EP 0 347 859 A2, a sterilization method for egg-shells is described. The sterilization method reported is unsuitable for eliminating the possible presence of pathogenic bacteria, spores, fungi and protozoans. The sterilization of egg-shell powder with

dry air at 120°C for about 1 hour is not suitable for effecting a safe reduction of pathogenic germs and to counteract a loss in active ingredients due to too high temperatures. An increase in temperature at > 80°C, especially in the range of ≥ 150°C, for more than 1 hour destroys the biologically present carriers with membrane passage ability for an effective transport of minerals in compact and spongy substances. Following this thermal exposure, the egg-shell powder exhibits the biological effects of calcium carbonate with respect to the ^{45}Ca incorporation rate.

Various formulations comprising egg-shell powder have been examined in US 3 558 711, especially in rats with topical application on open wounds. An improved wound healing has been achieved as compared to the control animals. In this document, no suitable sterilization method is reported which would not effect the therapeutic effectiveness of the egg-shells. An oral application of egg-shell powder is not intended.

In Chemical Abstracts, vol. 117, 1992, Ref. 33411x, egg-shell powder is processed into cosmetic preparations under the action of lactic acid. The calcium lactate products thus generated are embedded in a protein film. This lactate emulsion is processed into a cosmetic cream. In much the same way as preparation 1 (SU 1754 104), it only contains particular fractions of egg-shell which are topically applied.

In addition, there has been many decades of experience in the preparation of speciaties, in particular in the sterilization without activity losses, namely: if a temperature of 80°C is exceeded in the sterilization of the egg-shell, then the biological carrier with membrane passage ability for the minerals is destroyed, so that the activity of the thermally destroyed product corresponds to that of calcium carbonate with respect to the ^{45}Ca incorporation rate. On the other hand, this temperature alone is not sufficient to completely free the porous, heat-insulating raw material egg-shell/egg-shell powder from pathogenic bacteria, spores, fungi and protozoans the presence of which is

to be expected due to faecal contamination, especially when in addition the storage conditions are unfavorable.

According to the invention, it has been found that the use of putamen ovi surprisingly has advantages over the use of pure calcium carbonate in various conditions of disease. However, a particular problem in the use of putamen ovi as a medicament is to provide a standardized sterile medicament having a defined grain size.

In a first embodiment of the present invention, the above problem is solved by a method for the preparation of putamen ovi having a grain size of less than 0.1 mm wherein

a) egg-shells, especially from *Gallus domesticus*, are washed with water or an aqueous solution containing disinfectants and/or tensides with stirring at room temperature or elevated temperature;
b) the egg-shells having been cleaned from contaminants are subjected to a germ count reduction process or sterilization process;
c) the egg-shells are dried; and
d) the egg-shells are crushed to the desired grain size following or during the drying.

In the first cleaning step, the cleaning is effected by washing once or repeatedly with purified water (aqua purificata), possibly with stirring. Batch sizes of 50 to 100 kg of egg-shells usually require about 250 l of water; cleaning agents, especially surfactants, may optionally be used. The suspended matter formed in the cleaning is drawn off, and the cleaned egg-shells are subsequently subjected to a germ count reduction or sterilization process.

Subsequent to the sterilization or optionally during the sterilization, the egg-shells are dried at elevated temperature. This especially involves the evaporation of the water contained in the pores. Particularly preferred drying methods include vacuum

drying or freeze-drying as well as drying at increased temperatures.

By means of the present invention, it is possible to prepare putamen ovi having a defined grain size in which a major portion of the biologically active materials contained in the egg-shell in addition to calcium carbonate are conserved.

In the prior art, a number of germ count reduction processes or sterilization processes are known. Accordingly, in a particularly preferred embodiment of the present invention, said germ count reduction process or sterilization process is selected from autoclave treatment, hot air drying, tyndallization, treatment with ionizing or non-ionizing radiation, and gas sterilization.

Particularly preferred according to the present invention is hot air drying at a temperature above the boiling point of water, especially at a temperature of at least 150°C, for at least 3 hours in which sterilization and drying are conjoined in one process step.

Then, subsequent to the drying or during the drying, the egg-shells are crushed to the desired grain size of less than 0.1 mm. It is particularly preferred according to the present invention to crush the dried egg-shells with a grinding disk followed by screening with a mesh size of 0.1 mm x 0.09 mm.

Thus, depending on the grinding aggregate used, it is possible to provide putamen ovi having a defined grain size while essentially conserving the active companion substances.

Accordingly, a further embodiment of the present invention is putamen ovi obtainable by a method as defined above and having a grain size distribution of 35% by weight of > 0.05 mm and 65% by weight of < 0.01 mm.

The putamen ovi thus provided can be processed, using per se known adjuvants and vehicles, into a medicament which may be employed for various pathologic calcium deficiency conditions.

In particular, it has been found that a natural substance therapeutic agent thus obtained contains minor amounts of other essential minerals in addition to calcium, such as iron, fluorine, potassium, silicic acid, magnesium. In addition, it contains biologically generated active compounds, such as enzymes, porphyrin, sterols, vitamin D_3, along with the biologically important trace elements copper, molybdenum, selenium and zinc.

The presence of calcium in a biologically bound form so to speak represents a carrier function for a very effective resorption from the intestine into the blood. Good resorption of the minerals, the organic active compounds and the trace elements by the organism is a precondition for the clear effectiveness of putamen ovi in bone diseases and concomitant anemia.

The biologically important trace elements promote the development of a sound bone system and beneficially affect disturbed bone metabolisms.

With bone fractions, a significant shortening of the healing is achieved which is caused by a quicker callus and bone formation.

Osteopenia (loss in bone tissue) is not a disease but the age-dependent destruction of bone tissue which begins about from the 30th year of life and may amount up to 1.5% per year. Thus, until the 70th year of life, a loss of about 1/3 of the bone mass occurs without danger to the bone skeleton.

Upon insufficient calcium supply, in calcium resorption disorders or metabolic disorders, however, the organism withdraws calcium from the bones. Such withdrawal leads to a reduction in the bone substance. Not all bony organs are equally endangered. The spongy bones of the vertebral bodies (spinal column) are attacked first,

the tubular bones of arms and legs only later. Therefore, the spinal column is particularly in danger with the risk of spine deformation. The muscles try to counteract such changes in the spinal column. The additional muscular action sooner or later results in muscular pain. Therefore, muscular pain is present in osteoporosis.

Therefore, the putamen ovi to be obtained according to the invention is especially useful in the treatment of bone marrow development disorders, bone marrow dysfunctions, dyshematopoiesis, osteopenia, osteoporosis, dental build-up disorders, spasmophilia, bone fractions, callus formation and calcium deficiency symptoms during growth, pregnancy, postmenopause and nursing period.

The following examples serve to illustrate the invention without limiting the scope thereof.

Example 1

Preparation of putamen ovi.

Fifty kilograms of egg-shells from *Gallus domesticus* were washed 5 times with 250 l of aqua purificata at 60°C, the suspended matter being drawn off after each washing. The cleaned egg-shells were dried in a hot-air sterilizer at 165°C for 4 hours for germ count reduction and sterilization.

The dried egg-shells were ground with a pin-disk mill and separated with a screen having a mesh size of 0.1 mm x 0.09 mm.

Putamen ovi was obtained thereby having the following grain size distribution:

> 0.05 mm	35% by weight; and
< 0.01 mm	65% by weight.

Using per se known vehicles and citric acid, coated tablets were prepared which contained 440 mg of micronized putamen ovi, corresponding to 160 mg of calcium ions. The amount of citric acid was 1.07 mg.

Clinical tests:

With the coated tablets prepared above, clinical tests were performed in which the reduced bone density was examined with 41 female patients in the postmenopause.

The coated tablets were administered 3 times a day in the course of 304 days. An increase in bone density in the total universe of 9.4% after 304 days (as an average) was established. The test results are derived from repeated osteodensitometric determinations the follow-ups of which were additionally supplemented by the detection of the biochemical markers of the bone destruction (osteoclast activity) and activity of bone formation (osteoblast activity).

The bone mineral density measurements were performed by means of quantitative digital radiography (Hologic QDR-1000 TM bone densitometer) on the lumbar vertebrae 1-4 wherein the result of the bone mineral density calculation is expressed as density in gram of calcium hydroxyapatite/cm^3.

In this osteodensitometric procedure, the measuring value for the absorption in the soft-tissue coat was eliminated so that the bone mineral content could be determined without soft-tissue error. The overall bilance on the basis of 41 follow-ups showed a clear increase in the bone mineral density by 9.4% (from 78.1% to 85.5%).

In a group A which had been administered putamen ovi for less than 200 days with the dosage mentioned above, the bone density already increased by an average of 5.5%. In a group B which had been administered putamen ovi for less than 300 days with the

dosage mentioned above, the measuring value increased to 7.2% while in a group C which had been administered putamen ovi for more than 300 days with the dosage mentioned above, the measuring value increased to the 9.4% mentioned. These results showed a clear dose-effect relationship. While in a first group X, the value increased by 6.9% upon application of 3 times one coated tablet of putamen ovi, in another group Y which had been administered 2 coated tablets of putamen ovi 3 times a day, a bone density increase of 10.9% was found.

It has been found that the increase in bone density was less than 5% with 12 of the patients examined, between 5 and 10% with 18 patients, while this value was above 10% (+ 15.5%) with 11 patients. Thus, the responder rate may be assumed to be about 70%. No patient had a bone density after the end of the therapy which was below that of the initial examination.

By means of the present invention, it is evidently possible to generally counteract a bone density reduction of the spinal column in female patients in the postmenopause, even without a further substitution with osteoporosis therapeutic agents currently available commercially.